Plasticity in newt metamorphosis: the effect of predation at embryonic and larval stages

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SUMMARY
1. Some organisms under variable predator pressure show induced antipredator defences, whose development incurs costs and may be associated with changes to later performance. This may be of especial relevance to animals with complex life histories involving metamorphosis.
2. This study examines the effect of predation environment, experienced both during embryonic and larval stages, on palmate newt (Triturus helveticus) metamorphosis. Newt eggs were raised until hatching with or without exposure to chemical cues from brown trout (Salmo trutta), and larval development was monitored in the presence or absence of the cues.
3. Exposure to predator cues during the embryonic stage resulted in higher growth rates at the larval stage, reduced time to metamorphosis and size at metamorphosis. Metamorphs also had narrower heads and shorter forelimbs than those from predator-free treatments. In contrast, exposure to predator cues during the larval stage did not affect metamorph characteristics.
4. These results indicate that developing embryos are sensitive to predator chemical cues and that the responses can extend to later stages. Reversion of induced defences when predation risk ceased was not detected. We discuss the possible adaptive significance of these responses.

Keywords: amphibians, growth rate, induced defences, predator cues, reversibility

Introduction
Predation is one of the strongest selective forces in nature and has important effects on prey individuals, populations and communities (Sih et al., 1985; Lima, 1998). Organisms can counter predation risk by the development of behavioural, morphological, physiological, or life-historical defences (Kats & Dill, 1998). These may be phenotypically fixed (constitutive) or induced by cues associated with predation pressure (inducible) (Harvell, 1990). Constitutive defences, either structural or chemical, are characteristic of organisms exposed to constant predator pressure, whereas induced defences are often associated with fluctuating predation risk (Thompson, 1991; Clark & Harvell, 1992; Schlichting & Pigliucci, 1998). Induced defences allow individuals to respond to predator cues with dynamic changes in phenotype, avoiding the maintenance of defences when predators are not present (DeWitt, 1998). In this scenario, induced defences are expected to evolve preferentially in organisms exposed to high environmental variability, in such a way that a phenotype induced under particular conditions experiences higher fitness in those conditions than alternative phenotypes, but lower fitness than the alternative phenotypes in other conditions (Clark & Harvell, 1992; Schlichting & Pigliucci, 1998; Van Buskirk & Relyea, 1998; Relyea, 2002).

Fitness costs associated with the development of induced phenotypes are complex and include the cost
of maintaining sensory mechanisms for the detection of predation risk, as well as production and maintenance costs of the induced responses themselves (DeWitt, 1998; DeWitt, Sih & Wilson, 1998; Relyea, 2002). The maintenance of phenotypic plasticity may also affect individual fitness via genetic costs (linkage, pleiotropy, epistasis) or by causing developmental instability (DeWitt et al., 1998). These costs are usually manifested as slower growth, life-history modifications or lower competitive abilities (Sih, 1987; Lima & Dill, 1990; Lima, 1998). The existence of such costs and the lower fitness experienced in alternative environments explains the maintenance of plastic strategies instead of the fixation of specific antipredator defences (Harvell, 1990; Harvell & Toltlarian, 1999).

Amphibians show a variety of induced defences both in embryonic and larval stages and provide a good model system for the study of the induction of antipredator defences throughout the life cycle (Alford, 1999). Early induction of antipredator responses is well documented in many amphibian species that exhibit modifications to the timing of hatching, and morphology and development of the hatchlings after exposure to predation risk (Sih & Moore, 1993; Warkentin, 1995; Laurila et al., 2002; Orizaola & Branya, 2004). Larval amphibians also exhibit a number of behavioural and morphological modifications in the presence of predators, such as reduced activity, spatial avoidance, or relatively deeper tails and smaller bodies compared with non-exposed larvae (Anholt, Werner & Skelly, 2000; Van Buskirk & Schmidt, 2000; Van Buskirk, 2002b; Relyea, 2003b). These plastic responses increase individual survival when predators are present, but are also associated with reductions in growth and development in absence of predators (Smith & Van Buskirk, 1995; Van Buskirk & Relyea, 1998; Relyea, 2001b). Despite these well-documented cases, only a few studies have investigated antipredator defence mechanisms in consecutive ontogenetic stages of the amphibian life cycle (Van Buskirk, 2002a; Relyea & Hoverman, 2003). In particular, the potential reversibility of antipredator defences and the existence of mechanisms that compensate the effects of predators through the amphibian life cycle are poorly known.

In amphibians, metamorphosis is a crucial point that represents a change from an aquatic and relatively sedentary larval stage, to a terrestrial and more mobile stage (Wilbur, 1980; Alford & Harris, 1988), and several studies have shown changes in timing and size at metamorphosis, and in the morphology of metamorphs exposed to predation pressure (Laurila, Kujasalo & Ranta, 1998; Merila et al., 2000; Van Buskirk & Schmidt, 2000; Relyea, 2001b). In the present work we examined how exposure to predator cues at two consecutive stages of the life cycle affected metamorphosis in the palmate newt (Triturus helveticus Razoumowsky). In particular we tested whether the exposure of newt embryos and larvae to fish chemical cues could modify the time to reach metamorphosis, the larval growth rate, as well as the size and morphology of newt metamorphs. We also analysed the potential reversibility of predator-induced defences at both stages.

Methods

Our experiment involved palmate newt (T. helveticus), for which time to metamorphosis is under strong selection because of the conflicting demands of reducing exposure to predators and avoiding the desiccation of the larval environment. Several studies have reported behavioural and morphological plasticity in T. helveticus in response to predation pressure. For example, T. helveticus larvae reduced their activity and increased refuge use when exposed to direct trout presence (Orizaola & Branya, 2003), and reduced size, growth, and body mass just before metamorphosis when confronted with dragonfly predators (Van Buskirk & Schmidt, 2000).

The animals used in this study originated from 11 gravid females collected in lakes and ponds of Central Asturias (Northern Spain) during the 1999 breeding season. Females were housed in the laboratory and allowed to lay eggs in natural vegetation for several days. Every day, half of the eggs laid by each female were randomly assigned to each predator environment. Eggs were placed in 300-mL plastic bottles, filled with 0.25 L dechlorinated tap water or predator conditioned water, using different bottles for every female and day. A total of 50 bottles per treatment were used (nº of eggs per bottle, mean ± SE = 7.32 ± 0.22). During embryonic development water was changed daily in both treatments until hatching ceased (time to complete hatching, mean ± SE = 19.73 ± 0.29 days).

The larval part of the experiment started a few days after hatching and consisted of an orthogonal design
in which presence and absence of trout cues during the embryonic stage was crossed with presence and absence of trout cues during the larval stage. Before the start of this part of the study, all the hatchlings were maintained in an environment without predator signals. For each group, we selected 50 larvae at stage 14 of the Watson & Russell (2000) developmental series, with similar size (snout vent length-SVL) among groups ($F_{3,196} = 0.284$, $P = 0.836$). Larvae came from a pool of hatchlings, which allow us to be confident about obtaining a random sample from all sibships. Each larva was individually maintained in a 50-mL plastic bottle containing 35 mL of either dechlorinated tap water or predator conditioned water, changed daily. Every second day, larvae were fed live zooplankton (cladocerans and copepods) and frozen chironomid larvae. A small stone was placed in each bottle when larvae started to reabsorb their gills, to provide a perching place. The bottles were inspected daily for metamorphs (total gill resorption), which were removed, weighed and filmed with a closed circuit television (CCTV) Panasonic (Yokohama, Japan) camera to obtain a dorsal image. Six morphological traits of the metamorphs were then measured by means of image analysis software: head length, head width at eye level, head width at gill level, trunk length, trunk width at hindlimb legs and forelimb length (Fig. 1). Growth rate for each larva was defined as (SVL at metamorphosis – SVL at the start of the larval part of the experiment)/days from hatching to metamorphosis. The experiment was carried out in a room held at constant temperature (17 °C during embryo development and 22 °C during larval development) and under a constant 12 : 12 h photoperiod. We used different temperatures during the embryonic and larval part of the experiment to develop the study under the temperature conditions experienced by amphibian larvae in ponds of the study area (see Álvarez & Nicieza, 2002). The position of all the flasks during both embryonic and larval stages was rotated every day to avoid any effect of micro-environmental differences within the room. Because of the partial failure of one treatment, only data from ten metamorphs per treatment were included in the analyses.

Brown trout (Salmo trutta L.) was used as predator for both embryonic and larval development. A pair of large S. trutta (23.5–29.5 cm fork length, $n = 12$, mean ± SE = 25.9 ± 1.3 cm) was maintained in a 90-L tank with dechlorinated and aerated tap water to generate conditioned water (predator treatment). Predators were replaced by new ones six times during the experiment and returned to their place of origin. A similar 90-L tank with dechlorinated and aerated tap water was used to provide unconditioned water (no predator treatment). Both tanks were located in a room held at constant temperature (10 °C). Water was introduced into the experimental room and used only after it reached the temperature selected for the treatments. To avoid faecal contamination of water, trout were food-deprived and conditioned water was collected only after several days of trout gut clearing.

A two-factor analysis of variance (ANOVA) was used to examine the effects of embryonic and larval environment on time to metamorphosis, which was ln transformed to achieve normality. The effects of embryonic and larval environments on growth rate, and mass and SVL at metamorphosis were analysed using a two-factor multivariate analysis of variance (MANOVA, with embryonic and larval environments as factors). To examine the predator effects on morphological characteristics of the metamorphs, we regressed the linear measurements of all individuals
against their mass at metamorphosis, using the residuals in a MANOVA (embryonic and larval environments as factors). After the MANOVAs, subsequent ANOVAs were used to analyse univariate effects for factors that exhibited significant treatment differences, using the sequential Bonferroni correction to adjust significance levels. Deviation from normality was tested with Shapiro–Wilk tests and homogeneity of variances with Bartlett–Box tests. The level of significance was set at \( \alpha = 0.05 \) for all tests.

**Results**

Time to metamorphosis was influenced by the embryonic \((F_{1,29} = 18.299, P < 0.001)\), but not by the larval environment \((F_{1,29} = 0.311, P = 0.581)\). Larvae that had been exposed to trout cues during embryonic development metamorphosed on average 16 days earlier than those reared without predator cues (Table 1; Fig. 2). Moreover, newt larvae exposed as embryos to trout cues were 4\% shorter (SVL) and their mass at metamorphosis was 12\% lower (Table 1; Fig. 2). The embryonic environment also affected larval growth rate, which was 16\% higher in larvae exposed to predator cues (Table 1; Fig. 2), and influenced several morphological traits of newt metamorphs; individuals reared with predator chemical cues during the embryonic stage metamorphosed with narrower heads and shorter forelimbs, and also had a marginally significant tendency towards smaller trunks (Table 2; Fig. 3). In contrast, the environment experienced during the larval stage did not significantly affect the characteristics of the meta-

**Table 1** Results of a two-way MANOVA on the effects of embryonic and larval environments on larval growth rate and metamorph size (mass and SVL at metamorphosis) of palmate newt, and ANOVAS on the significant main effect

<table>
<thead>
<tr>
<th>MANOVA</th>
<th>d.f.</th>
<th>Wilk’s ( \lambda )</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonic environment</td>
<td>3,27</td>
<td>0.559</td>
<td>0.001</td>
</tr>
<tr>
<td>Larval environment</td>
<td>3,27</td>
<td>0.869</td>
<td>0.279</td>
</tr>
<tr>
<td>Embryonic × larval</td>
<td>3,27</td>
<td>0.855</td>
<td>0.232</td>
</tr>
</tbody>
</table>

| ANOVA on embryonic      | d.f. | \( F \)-value | \( P \)-value |
| environment effect      |      |              |              |
| Mass at metamorphosis   | 1,31 | 8.159        | 0.007*       |
| SVL at metamorphosis    | 1,31 | 10.262       | 0.003*       |
| Growth rate             | 1,31 | 16.629       | <0.001*      |

*Significant after sequential Bonferroni adjustment.

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**Fig. 2** Mean (+SE) time to reach metamorphosis, mean mass at metamorphosis, snout-vent length, and growth rate for newts that were incubated in predator and predator-free treatments, and reared in the presence or absence of predator chemical cues during the larval period.
morphs, although mass and SVL at metamorphosis tended to be lower (10 and 3%, respectively) in the predator treatment (Fig. 2).

Discussion

Environmental conditions experienced by animals during their development can induce plastic responses (Schlichting & Pigliucci, 1998). In particular, early environmental conditions (food, temperature, competition, predation) have been demonstrated to affect later performance both in invertebrates (e.g. Derlinger, 1981; Pechenik, Wendt & Jarrett, 1998) and vertebrates (e.g. Lindström, 1999; Relyea, 2001a; Räsänen, Laurila & Merilä, 2002). Moreover, several studies have shown that antipredator defences are induced preferentially in the earlier stages of development (e.g. Krueger & Dodson, 1981; Harvell, 1990; Brönmark & Miner, 1992; Arnqvist & Johansson, 1998). Accordingly, exposure to predator chemicals cues during embryonic development was associated in our experiment with earlier metamorphosis at a smaller size in T. helveticus. In contrast, we did not detect significant effects of predator environment experienced during the larval stage on the characteristics of newt metamorphs.

Several factors could explain the effects of the embryonic environment on later phases of newt development. The nature of the induced morphological changes, the probability of change of the environmental conditions and the growth mode of the organism (modular or not) have been signalled as factors conditioning the strength and reversibility of induced defences (Relyea, 2003a). The nature of induced antipredator responses can be influenced by the time of exposure to predators, as early phenotypic induction may put individuals on developmental trajectories that can have effects on phenotypes later in ontogeny and can compromise the reversal of the induced defences (Pechenik et al., 1998; Lindström, 1999; Stamps, 2003). The spatio-temporal persistence of the predator can affect the predictive value of actual predator presence, and thus may also affect the development and maintenance of induced defences. Predatory fish have a constant presence in permanent aquatic environments, and for this reason newts exposed to fish chemical cues during their embryonic stage could use these signals as a good indicator of fish presence. Contrary to our study, previous studies in which induced defences of amphibians reverted when predator pressure ceased, exposed anuran tadpoles (Rana temporaria Linneo and Hyla versicolor LeConte) to predatory dragonfly larvae (Van Buskirk, 2002a; Relyea, 2003a). Fish and dragonflies differ in many traits. For instance, the presence of dragonfly predators is temporal in aquatic environments, because they metamorphose and leave the ponds; for this reason their presence during earlier stages of the amphibian life cycle may not be a good indicator of their presence in later stages. Seasonal variability in dragonfly predation risk could be an explanation for the reversibility of induced defences found in these studies, as the existence of a high probability of change in environmental conditions is one of the main factors under which phenotypic reversibility should be favoured (Relyea, 2003a).

In our experiment, exposure to predator cues during the embryonic stage induced higher growth rates relative to controls during the larval period. However, exposure to predator cues during the larval stage did not affect T. helveticus growth rate, contrary to the results of previous studies on other amphibian species (Relyea, 2000; Van Buskirk, 2000). In natural habitats, the predator regime and especially fish presence seems to be relatively consistent within ponds over a single season (Van Buskirk & Relyea, 1998), so early determination of growth rates, even at the embryonic stage, could be adaptive if higher growth rates are favoured when predation risk is high. The acceleration

Table 2 Results of a two-way MANOVA on the effects of embryonic and larval environments on morphology of palmate newt metamorphs, and ANOVAs on the significant main effect

<table>
<thead>
<tr>
<th>MANOVA</th>
<th>d.f.</th>
<th>Wilk’s λ</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonic environment</td>
<td>6,24</td>
<td>0.478</td>
<td>0.004</td>
</tr>
<tr>
<td>Larval environment</td>
<td>6,24</td>
<td>0.679</td>
<td>0.124</td>
</tr>
<tr>
<td>Embryonic × larval</td>
<td>6,24</td>
<td>0.666</td>
<td>0.105</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ANOVA on embryonic</th>
<th>d.f.</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>environment effect</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head length</td>
<td>1,31</td>
<td>0.840</td>
<td>0.366</td>
</tr>
<tr>
<td>Head width (eye level)</td>
<td>1,31</td>
<td>3.657</td>
<td>0.065</td>
</tr>
<tr>
<td>Head width (gill level)</td>
<td>1,31</td>
<td>9.503</td>
<td>0.004*</td>
</tr>
<tr>
<td>Trunk length</td>
<td>1,31</td>
<td>5.587</td>
<td>0.024</td>
</tr>
<tr>
<td>Trunk width (hindlimb level)</td>
<td>1,31</td>
<td>3.255</td>
<td>0.081</td>
</tr>
<tr>
<td>Forelimb length</td>
<td>1,31</td>
<td>9.377</td>
<td>0.004*</td>
</tr>
</tbody>
</table>

*Significant after sequential Bonferroni adjustment.
of growth might allow newt larvae to improve locomotory performance, to escape from gape-limited predators, and to quickly attain the minimum size for metamorphosis. Reduced larval period associated with early metamorphosis reduces the time when individuals are exposed to predation risk (Wilbur, 1980; Werner, 1986; Wilbur & Fauth, 1990).

Plastic responses frequently involve some cost for the organisms (DeWitt, 1998; DeWitt et al., 1998; Relyea, 2002). Specifically, antipredator responses have often been associated with costs in metamorphic traits that could cause negative effects on reproductive capability, terrestrial growth, or survival (e.g. Smith, 1987; Semlitsch, Scott & Pechmann, 1988; Beck & Congdon, 1999; Altwegg & Reyer, 2003). In the present work, early metamorphosis, mediated by the exposure to predator cues during the embryonic stage, was associated with a smaller size and the

![Fig. 3 Relative morphological measurements (residuals on mass at metamorphosis; mean ± SE) of head, trunk, and forelimb length for *Triturus helveticus* metamorphs. Newts came from eggs incubated in the presence or absence of predator chemical cues during the embryonic period, and subsequently reared in the presence or absence of predator chemical cues during the larval period.](image-url)
modification of several morphological traits of newt metamorphs. Embryos reared in predator-conditioned environments produced metamorphs with narrower heads and shorter forelimbs at metamorphosis. Narrow heads could be associated with a reduction in the adult’s prey size spectrum, prey capture success, and handling abilities (Zaret, 1980; see Braña, De la Hoz & Lastra, 1986 for T. helveticus larvae of the same population), whereas reductions in limb length could be related to lower locomotory performance as reported for other salamanders (Bennett, Garland & Else, 1989).

Costs associated with the production of inducible defences should be low enough to make possible the maintenance of plastic strategies, allowing the growth, survival and reproduction of the individuals. The main cost of small size and early metamorphosis could be a reduction in juvenile survival (Altwegg & Reyer, 2003). However, despite the associated costs, plastic responses in T. helveticus are probably maintained because in this species most of their growth to reach adult size occurs after metamorphosis (e.g. in this study T. helveticus metamorphs mass was <2% of adult female mass). Previous studies on other tailed amphibian metamorphs have also reported that when metamorph size is so small relative to the adult, size at metamorphosis is not related with size at first reproduction (e.g. Hemidactylium scutatum Temminck and Schlegel; O’Laughlin & Harris, 2000). After metamorphosis, juveniles that experienced high predator pressure and metamorphosed at small size could exhibit compensatory growth, growing at a higher rate in terrestrial habitats, thus balancing the costs associated with predation risk avoidance. In any case, more studies on consecutive stages of the amphibian life cycle are needed to provide a better knowledge of the effects that predation pressure may cause in later stages of the amphibian life-history, and special attention should be focussed on the existence of reversibility or compensatory mechanisms of the induced antipredator defences.

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